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Applicant:	Grabowski and Du	: Paper No:	
Serial No.	09/775,517	: Group Art Unit:	1651
Filed:	February 2, 2001	: Examiner:	Jon Weber
For:	LIPID HYDROLYSIS THERAPY FOR ATHEROSCLEROSIS AND RELATED DISEASES		

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

David Hui Ph.D., declares that:

1) I am a Professor of Pathology & Laboratory Medicine at the University of Cincinnati. I received a Ph.D. in Biochemistry from Indiana University in 1979. I have worked at the University of Cincinnati since 1987. My areas of expertise include utilization of cellular, molecular, and genetic approaches to explore the structure and function of lipid transport proteins, as well as their roles in normal physiology and pathophysiologic conditions such as coronary artery disease, diabetes and obesity, hyperlipidmia, and nutrient digestion and transport.

2) I am familiar with the Grabowski and Du lipid hydrolysis therapy patent application, which describes a method to diminish and/or eliminate atherosclerotic plaques, in mammals, through direct and indirect treatment of these plaques, *in situ*, using proteins and/or polypeptides that are capable of lipid removal, primarily through hydrolysis. The lipid hydrolyzing protein or polypeptide targets receptors in and/or on the cell which lead to uptake into the lysosome.

Compositions used for practicing this invention include lipid hydrolyzing proteins or polypeptides, and in particular, lysosomal acid lipase (LAL).

3) A hallmark of atherosclerosis is the deposition of cholesterol and cholesterol-rich macrophage foam cells in the arterial wall. Increased circulating levels of low density lipoproteins (LDL) as well as decreased level of high density lipoproteins (HDL) are major risk factors for premature atherosclerosis. The mechanism by which increased LDL level contributes to atherosclerosis is well established. The prolonged circulation of LDL results in their increased susceptibility to oxidative modification and the oxidized LDL can be internalized into macrophages by a non-regulated scavenger receptor mediated endocytotic pathway. The internalized oxLDL are delivered to the lysosomes where the cholesteryl esters associated with the lipoproteins are hydrolyzed by lysosomal acid lipase (LAL), the unesterified cholesterol liberated from this hydrolysis exits the lysosomes where it can be esterified into cholesteryl esters by acyl-CoA:cholesterol acyltransferase (ACAT). The cholesteryl esters are stored in the cytoplasm as lipid droplets and the accumulation of these droplets result in foam cell formation.

4) Cholesteryl ester droplets in the cytosol of macrophages are not static and constantly undergo hydrolysis by neutral cholesterol ester hydrolases (including hormone sensitive lipase, HSL) and re-esterification by ACAT. In the presence of cholesterol acceptors, such as HDL, in the extracellular milieu, the unesterified cholesterol can exit the macrophages to these acceptors and prevent intracellular cholesteryl ester accumulation and foam cell formation. However, when extracellular cholesterol acceptor is limiting, such as the case under low HDL conditions, the unesterified cholesterol is re-esterified by ACAT and intracellular cholesteryl ester deposition and foam cell formation ensue.

5) A recent study by Escary et al. (Escary J-L, Choy HA, Reue K, Wang X-P, Castellani LW, Glass CK, Lusis AJ, Schotz MC. Paradoxical effect on atherosclerosis of hormone-sensitive lipase overexpression in macrophages. *J. Lipid Res.* 1999;40:397-404) reported that mice with transgenic over-expression of HSL in macrophages displayed a 2.5-fold increase in atherosclerotic lesion size compared to wild type controls when both strains of animals were fed an atherogenic diet. Importantly, however, was the observation that under the atherogenic dietary conditions, both control and HSL-transgenic mice displayed increased VLDL/LDL cholesterol level and a concomitant decrease in HDL-cholesterol.

6) Escary attributes the atherosclerotic lesions observed in these animals to the decreased level of HDL upon feeding them an atherogenic diet. According to Escary, the unesterified cholesterol released from lysed cells accumulates in the arterial wall, and ultimately leads to cholesterol crystal deposition and more advanced atherosclerotic plaque. Therefore, based upon Escary, one would predict that in subjects with normal levels of HDL in circulation, the increase in macrophage cholesteryl ester hydrolytic activity would be atheroprotective, whereas in subjects with low HDL, the increase in macrophage cholesteryl ester hydrolytic activity would have an opposite effect and would promote atherosclerosis.

7) In my scientific opinion, I believe that the Examiner's interpretation of Escary is not wholly on point. I do not think that ACAT comes into the picture here, because if one inhibits ACAT, this will induce cell toxicity, which is bad. One

certainly does not want to increase ACAT either because this would promote foam cell formation and atherosclerosis.

8) In the Grabowski and Du patent application, LAL is used as the enzyme to promote cholesteryl ester hydrolysis. LAL and HSL have distinct intracellular location and presumed to have distinct functions in intracellular cholesterol homeostasis. Whereas HSL in a cytosolic enzyme, thus capable of participating in hydrolyzing the cytoplasmic cholesteryl ester droplets in macrophage to limit foam cell formation, LAL is a lysosomal enzyme that participates in liberating unesterified cholesterol from cholesteryl esters entering the cells through endocytosis.

9) It is well-known in the art that generally, direct enzyme administration (i.e., exogenous administration) does not work well because of rapid clearance from circulation.

10) Based upon Escary, for patients with low HDL, one would predict that LAL therapy would be counter-productive and would result in increase atherosclerosis similar to the HSL mice discussed by Escary. Thus, Escary teaches away from the Grabowski and Du invention in that one reading Escary would not be motivated to administer an exogenous LAL in order to treat atherosclerotic lesions.

9) I hereby declare that all statements made herein of my own knowledge are true and that all statements made on the information and belief are believed to be true; and further that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any parent issued therein.

David Rui, Ph.D. 1/28/2004